stabilities. Another observation apparent in the table is that the two ureases have greater activity when adsorbed on the Fe(bipy)$_3$-smectite as compared with the HDTMA-smectite, even though equal quantities of enzyme (1% by weight) are immobilized on the two organo-smectite complexes. This suggests easier access to active sites for the substrates.

The use of organo-smectite complexes for immobilization of enzyme systems has several advantages. First, by placing the proper organic cation on the mineral, one can create 'tailor-made' surfaces having a variety of kinds of interactions with proteins and enzymes in particular. Second, since clays possess large surface areas, a large amount of enzyme can be bound on a given quantity of clay mineral[9]. Thirdly, as reported above, different kinds of mineral-organic matrices may have different specificities regarding the activities of various adsorbed enzymes, perhaps giving information about the nature and location of the active sites in the enzyme. Finally, it seems likely that clay-organic complexes could be used as models for a variety of systems such as biological membranes, since surface properties such as hydrophobicity, polarity, and aromaticity can be created as desired. These systems may also be useful in the study of important natural processes such as the stabilization of soil enzymes and soil organic matter, and for industrial applications.

1 This work was partially supported by the National Science Foundation under Grant No. CHE-8306583. Journal Article No. 11603 of the Michigan Agricultural Experiment Station.
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0014-4754/85/121564-03$1.50 + 0.20/0

Effects of acetaldehyde and/or ethanol on neutral amino acid transport systems in microvillous brush border membrane vesicles prepared from human placenta

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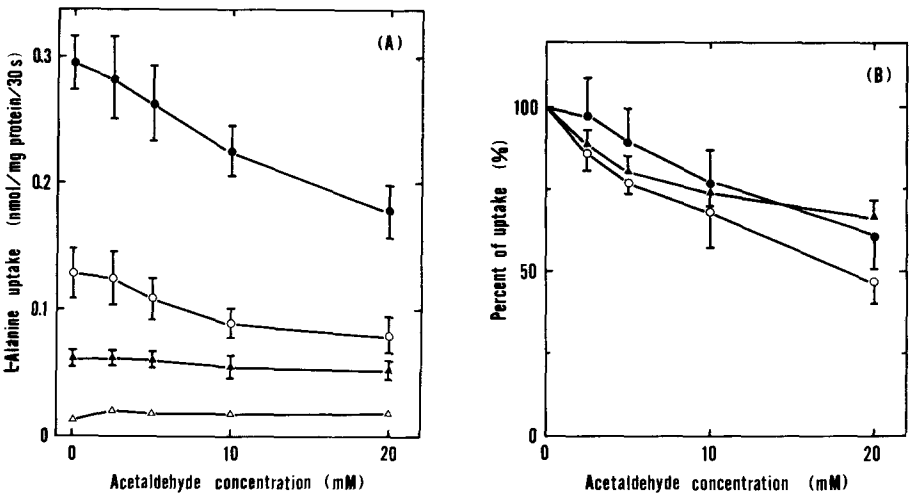
Summary. At 20 mM of acetaldehyde, the activities of three transport systems of L-alanine distinguished by the difference in their cation dependence, namely 1) Na^+-specific, 2) Li^+-dependent, and 3) Na^+-independent systems, were significantly reduced in a similar manner. Only the Li^+-dependent system was selectively inhibited at toxic concentrations of acetaldehyde and ethanol.
Key words. L-Alanine; transport system; membrane vesicle; human placenta; acetaldehyde; ethanol.

It has been well documented that the fetal alcohol syndrome (FAS) results from the adverse effects of maternal ethanol consumption on fetal growth and development[1,2]. Principal features of FAS are central-nervous-system dysfunctions, facial characteristics, and growth deficiencies. The pathogenesis of FAS has been currently investigated and reviewed[3-5], considering various factors such as the direct mutagenic effects of ethanol or acetaldehyde, the inhibition of protein synthesis, the alteration of neurotransmitter or hormonal balance, and the inhibition of placental transfer of nutrients. There are several reports that impairment of the placental amino acid transport is one of the causes of the intrauterine growth retardation[6-8].

Although the effects of acetaldehyde or ethanol on amino acid transport have been studied in some organs and cultured cells[9-12], no investigations of direct actions on the transport systems have been reported so far because of possible complications arising from unknown internal compartmentalization and intracellular metabolism. Membrane vesicles provide a simplified system, in which a transport process can be studied under well-defined conditions dissociated from intracellular components[13], and in addition, known magnitudes and polarities of chemical or electrochemical driving forces can be imposed across the membrane[13,14].

In our previous report, it was demonstrated that the Li^+-dependent uptake of L-alanine, presumably by the 'ASC' system, in placental membrane vesicles was selectively inhibited by ethanol[15]. The present communication concerns the direct effects of acetaldehyde and/or ethanol on the neutral amino acid transport systems using 0.1 mM L-alanine and the microvillous brush border membrane vesicles prepared from human placenta.

Materials and methods. L-[^{14}C(U)]-Alanine (174 mCi/mmole) was purchased from New England Nuclear Corp. (Boston, MA); acetaldehyde (99% pure) from Merck (Darmstadt, Germany); ethanol (99.5% pure) from Katayama Chemical Ind. (Osaka, Japan). All other chemicals were of reagent grade. Placentae were obtained at delivery from normal full-term vaginal deliveries. Microvillous brush border membrane vesicles were prepared in mannitol buffer consisting of 0.3 M mannitol and 10 mM Tris-HCl buffer, pH 7.4, by a modification[14] of the method described by Smith et al.[16]. The membrane vesicles were stored at 0 °C until used (within a few days). The experiments on uptake were performed according to the following procedures. Incubations were initiated by adding the membrane vesicles to the incubation medium and carried out for 30 s at 25 °C in a total volume of 100 µl containing 80–100 µg of vesicle protein, 0.1 mM L-[^{14}C(U)]-alanine (10 mCi/mmole), 100 mM NaCl, 100 mM mannitol and 10 mM Tris-HCl buffer, pH 7.4. The addition of acetaldehyde and/or ethanol to the incubation medium was done just before the initiation of uptake in order to avoid evaporation of the substrate(s). In some experiments NaCl was replaced isoosmotically by LiCl or mannitol. Uptake was terminated by the addition of 1 ml ice-cold 10 mM Tris-HCl buffer, pH 7.4, containing 0.8 M NaCl (wash buffer), followed by immediate filtration through a Millipore filter (HAWP; pore size, 0.45 µm). The filter was washed twice 5 ml ice-cold wash buffer. Radioactivity of the dried filter was measured in toluene scintil-



Effects of acetaldehyde concentration on the rate of L-alanine uptake. Each value is the mean ± SEM (bars) of five experiments from different placentae. Where no SEM is indicated the values fall within the limits of the symbol. *A* L-Alanine uptakes under four conditions: 100 mM Na^+-gradient (●), 100 mM Li^+-gradient (○), cation-free medium (▲), and cation-free medium supplemented with 20 mM L-leucine (△), which represents the simple diffusion as described in the text. *B* L-Alanine uptake characterized by the difference in cation dependence. Na^+-specific uptake (●), Li^+-dependent uptake (○), and Na^+-independent uptake (▲). In each component the L-alanine uptake in the absence of acetaldehyde is designated as 100% in each placenta. For detailed explanation refer to the text.

lator with a Beckman model LS-9000 liquid scintillation system. Correction for non-specific retention by the filter was made by subtracting zero-time radioactivity. Protein was measured by the method of Lowry et al.[17], using bovine serum albumin as a standard. Statistical analysis was made by the matched t-test.

Results. Figure 1A represents the effects of increasing concentrations of acetaldehyde on the L-alanine uptake under four conditions; 1) 100 mM Na^+-gradient and 2) 100 mM Li^+-gradient toward the vesicles, 3) cation-free medium and 4) cation-free medium supplemented with 20 mM L-leucine. The uptake process in condition 4 represents simple diffusion, because in the cation-free medium the neutral amino acids are transported by the 'L' system and simple diffusion[18,19], and the uptake by the former is completely inhibited by an extremely high concentration of L-leucine[18]. A considerable inhibition by acetaldehyde of the L-alanine uptake was observed both in the Na^+- and the Li^+-gradients, and the uptake in the cation-free medium was slightly decreased. On the other hand, the simple diffusion was enhanced 1.5 times by acetaldehyde at a concentration of 20 mM (0.0136 to 0.0198 nmol/mg protein/30 s).

We tentatively classified the process of L-alanine uptake into three components, characterized by the difference in cation dependence. The Na^+-specific uptake was calculated by subtracting the uptake in the Li^+-gradient from that in the Na^+-gradient. The uptake in the Li^+-gradient minus that in the cation-free medium represented the Li^+-dependent uptake. The Na^+-independent uptake was defined as the uptake of L-alanine in the cation-free medium which was subjected to inhibition by 20 mM L-leucine. All three components, which were expressed as a percent of the uptake in the absence of acetaldehyde, were increasingly inhibited by acetaldehyde as its concentration was increased (fig. B). At 20 mM of acetaldehyde, the Na^+-specific, Li^+-dependent, and Na^+-independent uptakes were significantly reduced, to 60.5%, 47.0% and 66.0% of the respective values in the absence of acetaldehyde ($p < 0.01$, $n = 5$), but no statistically significant difference was observed between the three components.

In order to see the effects of acetaldehyde and ethanol in the toxic concentration range (refer to discussion), L-alanine uptake was examined at 50 μM of acetaldehyde and/or 87 mM of ethanol (table). Neither acetaldehyde nor ethanol alone gave any significant effect on all three components of the L-alanine uptake. However, the combination of both inhibited selectively only the Li^+-dependent uptake as much as by about 30%, without affecting the other two uptake processes.

Discussion. This study demonstrates the effect of acetaldehyde and/or ethanol on L-alanine transport systems which can be distinguished by the difference in their cation dependence. L-Alanine was selected as a radiolabeled substrate because its membrane transport has been well described[14,18].

Most neutral amino acids are transported by several transport systems, and three distinct systems are recognized as 'A', 'ASC', and 'L' systems in Ehrlich cells[20]. The 'A' system is Na^+-dependent and the 'L' system Na^+-independent. A second Na^+-dependent transport system, termed the 'ASC' system, is distinguished from the 'A' system by its greater reactivity with such amino acids as L-alanine, L-serine, and L-cysteine, and also by its intolerance of N-methylation of substrates[18-20]. The tolerance of Li^+ substitution for the 'ASC' system is not well established yet, but Edmondson et al.[18] reported that the Li^+-dependent uptake of L-alanine by rat hepatocytes was mediated by the 'ASC' system. We also demonstrated that the Li^+-dependent L-alanine uptake by human placental membrane vesicles was not inhibited competitively by 2-(methylamino)-isobutyric acid (unpublished data), which was a specific substrate for the 'A' system[19,20]. Thus, it is considered that the Na^+-specific and Li^+-dependent uptakes are mediated, respectively, by the 'A' and 'ASC' systems, and the Na^+-independent uptake by the 'L' system.

Our earlier study showed that a high concentration (0.87 M) of ethanol caused reduction of the Li^+-dependent L-alanine uptake by placental membrane vesicles[15]. To our knowledge, however, the direct action of acetaldehyde, the major metabolite of ethanol, on neutral amino acid transport systems has not been documented so far. At 20 mM of acetaldehyde, a concentration about 100 times greater than the highest blood level (220 μM) reported with Oriental males having a history of intense facial flushing after alcohol ingestion[21], which can never be reached in vivo, the activities of the L-alanine transport systems were significantly inhibited and the 'ASC' system appeared to be most sensitive (fig.). In contrast to ethanol no statistically significant difference

Effects of acetaldehyde and ethanol on three transport systems for L-alanine discriminated by the difference in cation dependence

	Percent of uptake		
	Na^+-specific	Li^+-dependent	Na^+-independent
Control	100	100	100
Acetaldehyde (50 μM)	112 ± 11.9	86.4 ± 4.57	116 ± 8.90
Ethanol (87 mM)	99.0 ± 7.07	96.3 ± 6.15	89.4 ± 6.80
Acetaldehyde (50 μM) + ethanol (87 mM)	110 ± 5.55	68.9 ± 6.26*	94.8 ± 4.99

Each value represents the average ± SEM for five placentae; *$p < 0.01$.

was observed between the three transport systems. The reason for this difference in action between the two agents remains unclear.

Blood ethanol concentrations ranging from 2 to 5.4 g/l are found among sober alcohol users seen in an emergency room[22], and levels as high as 7.8 g/l (about 170 mM) have been reported[23]. It has also been shown that acetaldehyde in blood ranges from 40 to 50 μM in alcoholic patients[24] and from 24 to 220 μM in the Oriental males mentioned above[21]. These observations indicate that the concentrations of acetaldehyde (50 μM) and ethanol (87 mM) used in the table are within the toxic range, but far below the lethal doses. The results presented in the table revealed that no inhibition of the 'A' system occurred at 87 mM of ethanol. This contrasts with the report by Dorio et al.[12], who described that in cultured rat liver cells the major inhibitory effects of ethanol (100 mM) exposure for 24 h were on the 'A' and 'N' systems. The discrepancy seems to be derived from the difference in methodology employed. The use of membrane vesicles has some advantages over studies at the cellular level; that is, the former provides well-defined conditions dissociated from intracellular components[13] and also makes it possible to measure the transport activity at known magnitudes and polarities of chemical or electrochemical driving forces across the membrane[13,14]. Thus, it is considered that our results are not complicated by the activities of Na^+, K^+-ATPase and protein synthesis, which are inhibited by ethanol[7,9].

Only the Li^+-dependent uptake, which was transported by the 'ASC' system, was significantly inhibited by the combination of acetaldehyde and ethanol (table). This observation is partly supported by the previous report that the plasma concentrations of alanine and serine, which are mainly transported by the 'ASC' system, are decreased by an ethanol diet in rats[25]. Thus, it is considered that acetaldehyde in combination with ethanol may add to the effects of ethanol. The selective inhibition of the 'ASC' system would appear to suggest a higher sensitivity of the transport protein and its surrounding membrane lipids to acetaldehyde-and-ethanol exposure. We speculate that the amino acid imbalance induced by the inhibition of a particular transport system may play a role in the pathogenesis of FAS in association with other factors such as zinc deficiency, the inhibition of protein synthesis and altered hormonal and neurotransmitter balance[3-5].

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0014-4754/85/121566-03$1.50 + 0.20/0

Quantitative differences in the pharmacological effects of (+)- and (−)-cathinone

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Summary. The optically pure isomers of cathinone were prepared by separating synthetic cathinone racemate and used to study central and peripheral effects of these indirect sympathomimetics in rats and guinea pigs. The (−)-isomer was significantly more potent than the (+)-isomer in stimulating locomotor activity whereas no difference was observed with respect to their cardiac effects. In analogy to observations with (+)- and (−)-amphetamine such variable isomer discrimination may be due to different stereoselectivities of amine uptake mechanisms in the target tissues.

Key words. (+)-Cathinone; (−)-cathinone; cardiac stimulation; locomotor activity.

Khat leaves *(Catha edulis)* are widely used as a stimulant in East Africa and the Arab Peninsula. Most of their pharmacological effects are explained by their content of (−)-cathinone ((S)-(−)-α-aminopropiophenone), an indirect sympathomimetic with amphetamine-like centrally stimulating properties[1,2]. The actions of cathinone have been studied primarily by applying the (−)-isomer or the racemic drug. (+)-Cathinone ((R)-(+)-α-aminopropiophenone), does not occur in the plant but is obtained during chemical synthesis of the compound. It has been found to be less potent than (−)-cathinone as a stimulant of locomotor activity in mice[3,4]. However, a quantitative comparison of the central and peripheral effects of the enantiomers is lacking.

Therefore, we decided to study the potencies of synthetic (+)- and (−)-cathinone in two model systems. The locomotor activity